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Upon assimilation of atmospheric nitrogen by fungi

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For several years the writer has been interested in the question of assimilation of atmospheric nitrogen by fungi. Experimental work was begun in 1907 upon *Fusarium Zeae*[1] at the suggestion of Professor J. B. Pollock, of the University of Michigan, to whom the writer is indebted for many timely suggestions and criticisms.

The fungus was cultivated in several kinds of organic and synthetic media with dextrose, cane sugar, glycerine, or potassium tartrate as the source of carbon. In all cases the fungus made a good growth when nitrogen was supplied in the form of a nitrate; if very small amounts of nitrogen were supplied, the growth was proportional to the amount of nitrogen; if no nitrogen was supplied there was no growth beyond the mere germination of the spore. In no instance did the most careful chemical analysis of the fungus felt and the medium in which it grew, show any increase in the amount of fixed or combined nitrogen.

These experiments led to similar experimental work with other species of fungi to determine whether any of them have the power to assimilate or fix atmospheric nitrogen, and to ascertain, if possible, why so many contradictory results have been obtained by different investigators. For example, Berthelot,[2] Puriewitsch,[3] Saida,[4] Froehlich,[5] and Latham[6] have reported positive results with *Aspergillus niger*, while Czapek,[7] Koch,[8] and Winogradski[9] give negative results for the same fungus.

Thus far, two species of *Penicillium*, *Aspergillus niger*, an *Alternaria*, and two species of *Fusarium* have been tried by the methods reported by Ternetz[10] and Froelich.[5] After the appearance of Latham's[6] work, in which she reported a very large increase in the amount of combined nitrogen in some cultures of *Aspergillus niger*, which had been supplied with large amounts of ammonium nitrate, a special series of experiments was tried with this fungus in a medium containing a large amount of ammonium nitrate.

In general, the method has been to grow pure cultures of the fungus in 100 c.c. of the solution in one-liter flasks. These flasks were arranged in a series with wash bottles of sulfuric acid and potassium hydroxid and connected by tubes through their rubber stoppers in such a way that air, freed from all combined nitrogen in the form of ammonia and oxids of nitrogen, could be drawn through the flasks by means of a filter pump. Controls were always used, some consisting of 100 c.c. of the medium without the fungus, others of cultures of the fungus in the medium to which enough combined nitrogen had been added to insure a good growth of the fungus. At the conclusion of the growing period chemical analyses were made of the cultures and controls to determine the mount of combined nitrogen in each.

In the chemical analyses different modifications of the Kjeldahl method were used. If it was certain that no nitrate was present, Gunning's modification was found to be the most satisfactory; if nitrates were present, the Gunning-Jodlbauer modification was used. When very small quantities of nitrogen were to be determined, as in the culture media in which no combined nitrogen has been put or in a very small fungus felt, the digestion was carried out in the usual way, made alkaline, then distilled and the distillate caught in a volumetric flask containing a small amount of dilute hydrochloric acid. The amount of ammonia was then determined by the colorometric method in which Nessler's reagent and Nessler's comparison tubes were used. When the amount of nitrogen was about 0.5 milligram or less, this method was found to be more satisfactory than the ordinary method by titration. There is also no danger of error by the carrying over of small amounts of the fixed alkali, during the distillation, for they would make little if any difference in the color.

In all cultures in which dextrose was used there was some growth. In the first series the titration method was used and the total nitrogen content of the several flasks was found to be 0.44–0.62 milligram. A blank with one gram of dextrose gave 0.42 milligram of nitrogen. In the next series, the colorometric method was used and all analyses of blanks and cultures in which cane sugar, glycerine, and potassium tartrate were used gave amounts of nitrogen between 0.30 and 0.37 milligram. At the same time analyses of blanks and cultures in which dextrose was used gave amounts of nitrogen between 0.44 and 0.47 milligram. From these results it is evident that although the dextrose contained a very small quantity of combined nitrogen to begin with, no nitrogen was assimilated or fixed in any of the cultures.

In the next series of experiments, cultures of two species of *Penicillium*, *Aspergillus niger*, an *Alternaria*, and another species of *Fusarium* were tried with dextrose (a new stock). In all the flasks there was a little growth. The analyses, again by the colorometric method, gave amounts of nitrogen between 0.53 and 0.60 milligram, with but a single exception. One culture of one *Penicillium* gave 0.88 milligram of nitrogen. Since, however, the growth of mycelium was no greater in this culture than in the others it was thought that the excess of nitrogen may have come from something which might have dropped into the flask during the preparation of the cultures or in the analysis.

In the experiments with *Aspergillus* (*Sterigmatocystis*) *niger* in which Latham's method was followed, the following results were obtained: A thick felt of fungus hyphae was formed in each experimental flask during the six-day growing period. Each fungus felt was carefully filtered, dried, weighed, and analyzed for its nitrogen content. The filtrate was also analyzed for its nitrogen. The total nitrogen content for three cultures was 155.8, 154.5, and 153.1 milligrams, respectively (average 154.5 milligrams), and for three control flasks 156.1, 154.4, and 151.3 milligrams, respectively (average 153.6 milligrams). The individual differences were probably due in part to the fact that the culture medium was measured out in an ordinary graduated cylinder. To test the efficiency of the method of analysis, a sample of ammonium nitrate was carefully weighed and analyzed. The computed amount of nitrogen which it should have contained was 177.92 milligrams; the analysis gave 176.9 milligrams. This was considered very close in view of the fact that ammonium nitrate is deliquescent.

Although these results are in harmony with the generally accepted notion that fungi do not have the ability to assimilate atmospheric nitrogen, the persistency with which reports of nitrogen assimilation by fungi appear demands attention and explanation. There are at least two very probable reasons for the positive results that have been reported by different investigators. The first is the old and often repeated one of experimental error. This reason or objection may properly be given, for it is a well known fact that the determination of nitrogen by the Kjeldahl method presents many difficulties even to those who are more or less expert in making such analyses. This statement will be borne out by an examination of the reviews and reports of controversies among agricultural chemists over nitrogen determinations. One instance may be cited. Barral reports that new block tin condensers were found to absorb considerable ammonia so that accurate results could be obtained only after they had been in use a certain length of time. Such a source of error might cause a whole series of controls to show on analysis a smaller amount of nitrogen than a like series of experimental cultures. When the analyses of six similar cultures in 50 c.c. of culture medium give amounts of nitrogen varying from a loss of 44.9 milligrams in one flask to a gain of 193.6 milligrams in another, it is not safe to assume that free nitrogen has been fixed even although the average for the six cultures may be higher than the average nitrogen content for six controls. The results for the individual cultures should be more nearly uniform or there should be a reasonable explanation for the great differences in the nitrogen content of the different flasks. The objection on the ground of experimental error has added weight when we consider that many of these experiments have been carried out by persons who are amateurs in chemical analysis.

The second reason, which does not seem to have been advanced as yet, is suggested by work such as has been done with *Penicillium* by Thom.[11] It is very possible that there are some strains or varieties of fungi which have the ability to use nitrogen while many other very similar strains do not have that ability. Different species may have been confused and used under the same name by different writers. Undoubtedly several species have been used under the name of *Penicillium glaucum*, and the same may be true with regard to other species of fungi. With the view of obtaining more definite data upon this problem, experiments are now under way in our laboratory to obtain as many as possible distinct strains of fungi by cultural methods and to test them as to their ability to use free nitrogen.

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